Turning points

Migrating to butterflies

Sean B. Carroll

Many of the stories that have appeared in this column recount the way in which the timely appearance of a paper catalyzed the pursuit of a new line of research. The experience is, happily, not limited to new papers. One of the greatest pleasures in research is to discover (or more accurately, to rediscover) a gem of a paper that has been in the literature for some time but whose findings or promise have not yet been realized.

The rediscovery of Mendel's work or the re-examination of Walcott's interpretations of the Cambrian fauna of the Burgess Shale are famous examples of this, but our more modest excursion from the comfortable confines of *Drosophila melanogaster* developmental genetics to the psychedelic world of butterfly wing patterns was also triggered by such a rediscovery.

In the early 1980s, I was prompted to work on the developmental genetics of *Drosophila melanogaster* by the conviction that the way to achieve an understanding of morphological evolution was through the genetic basis of animal form. Yet, for the next decade, I did not initiate any comparative or evolution-oriented work. In part, my hesitation was due to a sense that, because we had so much to learn about developmental regulatory mechanisms in model systems, comparative studies would be superficial at best. Part of the problem, however, was my failure to identify tractable models for such comparative work.

A casual conversation on a seminar visit at Duke University in 1991 turned things around. While hurrying across a parking lot to make my next appointment, Fred Nijhout asked me, quite innocently, whether the mechanisms we were studying that position bristles on the fruitfly could help explain what really interested him — the formation and diversity of butterfly wing color patterns.

"I don't know," was my lame answer but I promised Fred I would think about it. As I knew nothing about butterflies (I was not a collector as a kid because houseflies were about the most exciting fauna in Toledo, Ohio), I went to the literature to find out about their wing patterns. Much of what had been published, I learned, had been written by Fred.

I found a pair of papers written in 1980 that describe some remarkable manipulations of butterfly wing patterns [1,2]. The central interest was in eyespots, the variously colored concentric rings of pigmented scales that are used by Lepidoptera to avoid predation. Fred surmised that these rings were organized by a focal source at their center so he tested this idea by ablating small areas of the developing wing. Sure enough, the presumptive centers were necessary for the formation of the eyespots. More spectacularly, when Fred transplanted these centers to other sites on the wing, new eyespots were induced.

The eyespot center, or focus, was clearly a developmental organizer on a par with more famous regions or zones of amphibian embryos or vertebrate limb buds. Moreover, the organizer and eyespot patterns had evolved within this order of insects. So, Fred's experiments had identified two big questions in one sweep. First, what are the developmental mechanisms underlying the formation and activity of the focus? And second, how does a new organizer evolve, superimposed on the conserved general ground plan of an insect wing?

Fred's results had been known for a dozen years, but no molecular studies of the eyespot focus had materialized. This was our golden opportunity because, although we knew very little about butterflies, we were immersed in the genetic dissection of *Drosophila* wing development. We just used our knowledge from flies to take a look at how butterflies might do things similarly, or better yet, differently. We did, indeed, discover that eyespots were special and formed from unique patterning systems operating within the butterfly wing.

Wing walking: a fruit fly strolls across one of the butterfly's best works of development.

Our migration into butterfly wing development opened our eyes to many questions that could be pursued with these beautiful animals and served as our training ground for comparative approaches to more exotic creatures. It has fostered interdisciplinary and collaborative approaches to ecology, genetics, development and evolutionary biology that were not foreseen when we began. Our initial rediscovery of Nijhout's work has led to our rediscovery of classic work on mimicry, melanism, plasticity and other phenomena that beg for a developmental and genetic explanation and that frame our current agenda.

Most scientists will admit to occasional serendipitous discoveries.

In my case, it's possible that none of this would have happened if I hadn't had the luck to be in the right place (a parking lot in North Carolina) at a time when studies in *Drosophila* were far enough advanced to allow us to venture off onto roads less traveled.

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5000-year-old myelin: uniquely intact in molecular configuration and fine structure

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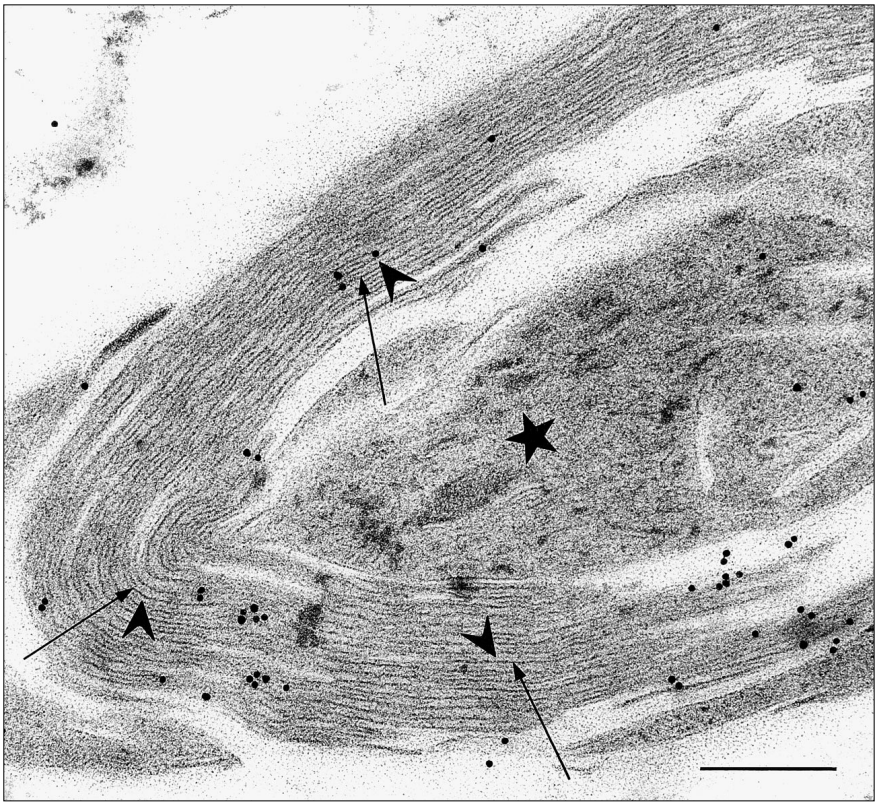
An almost intact, naturally mummified late neolithic human was discovered in 1991 at a glacial field in the Ötztal Alps. Not only the clothing and equipment of the 'Tyrolean Ice Man', but also the frozen mummy itself, appeared in good overall condition [1]. At the subcellular and molecular level, however, considerable decay was observed ([2]; we examined more than 50 tissue samples from all major organ systems, except for the heart and the urogenital system, data not shown). Here, we describe the one extraordinarily well preserved subcellular constituent in the Ice Man: the myelin sheaths. After millenia they still display outstanding structural and molecular integrity, a feature never before demonstrated from any ancient remains [3,4].

Our approach required only extremely small tissue pieces from this unique specimen. Autopsy samples of 1 mm^3 [5] were subjected to freeze-substitution [6], followed by post-embedding immuno-gold and lectin-gold labelling [7,8].

The femoral nerve and sympathetic trunk locally exhibited ultrastructural patterns strongly resembling myelin sheaths (see Figure 1): major dense lines alternate with intraperiodal lines, their periodicity being similar to present-day samples [9]. (Note that the Ice Man's cerebral cortex also showed recognisable myelin remains, but their chemical preservation was inferior to that of the femoral nerve; data not shown.)

The remarkable resistance of myelin to freezing and thawing is already known from experimental studies in which only minor alterations of myelin periodicity were observed [10]. One plausible interpretation relates to the specific chemical composition and biophysical properties of myelin. Low water content, with high proportions of lipids, together with the highly ordered configuration of the multi-lamellar myelin membranes may have limited ice nucleation. Note, however, that repeated freezing and thawing of the Ice Man during the preceding 5000 years (including the recovery,

Figure 1



Segment of myelinated femoral nerve fibre from the prehistoric Tyrolean Ice Man in cross-section. Immunolabelling is with anti-GalC [12] and visualization with 10 nm colloidal gold. Arrows indicate putative major dense lines, arrowheads indicate intraperiod lines, and the star indicates axon remains. The scale bar is 200 nm.

subsequent storage of the corpse at –6°C and sampling) also caused local structural breakdown and rearrangement of myelin components. This is indicated by nerve fibre remnants displaying disordered ultrastructure.

Interestingly, structural breakdown was not necessarily associated with severe chemical alterations: adipocere — an indicator for certain post-mortal transformations [11] — is almost absent in the Ice Man's peripheral nerves, in contrast to its predominance in his other tissues (data not shown) and in other glacier corpses; moreover, structurally disordered myelin could be stained specifically.

We revealed antigenic reactivity and lectin-binding capacity of major myelin constituents in the Ice Man's femoral nerve (see Figure 1). Moderate, though distinct, immunolabelling of both intact and disordered myelin was consistently obtained with antibodies recognising three groups of myelin constituents: galactocerebroside, sulfatide and other, structurally related, glycolipids (26% of total myelin lipid, detected with anti-GalC [12,13]); P0-glycoprotein (50% of peripheral myelin protein [14], antibody courtesy of H. Lassmann, Vienna); and myelin basic protein [15]. Reaction specificity was assessed by observing low background labelling on collagen fibrils and cells of the perineurium and endoneurium serving as internal negative controls, and by omitting primary antibodies or replacing them with irrelevant antibodies.

Myelin oligosaccharides bound the lectin RCA_{120}, confirming the presence of terminal galactosides as found, for example, on galactocerebroside and on the glycan chains of glycoproteins. Positive, though weaker, labelling with other lectins (WGA, HPA, and UEA-I) strongly suggested the presence of terminal and subterminal galactosides, fucosides and the polylactosamines forming intermediate links in the glycan structures. Furthermore, Con A, a sensitive indicator for disruption of these structures, did not react with myelin.

Together, as far as antigenic and lectin-binding sites of major myelin constituents are concerned, the amino-acid and sugar composition, and even the spatial arrangement of these components, appear remarkably well preserved. Thus, the molecular architecture of myelin in the peripheral nervous system of the Tyrolean Ice Man can be considered essentially undamaged; furthermore, we depicted structures probably representing intact fragments of myelin sheaths, features never before reported from ancient remains.

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